**Objectives**

**Methods**

**Linking 16S sequences from Shotgun Libraries to Bins & Amplicon Profiles**

Before attempting to link OTUs to bins, different approaches to read mapping, composition comparison and data normalization were tested using OTUs sequences derived from the full series of amplicon libraries collected at Mystic Lake. Comparable composition vectors were created by querying each amplicon sequence to against the shotgun metagenomic libraries. All amplicons were trimmed to 102 bp and the vast majority of reads were > 130 bp after quality control, but anything shorter was removed usisng seq-tk (). Bowtie2 was used with modified parameters to map amplicons to reads. The non-default flags used include ` --score-min 'C,0,-1'`, `--all`, and `--no-unal`. Together, these require that all instances in which the entire query sequence is matched to the reference or its reverse compliment.

The resultant SAM files were parsed by a custom script. During parsing it was recognized that two pairs of amplicons ('seq1799'/'seq50099' and 'seq83'/'seq86424') were aligning to the same reads, offset by a single nucleotide. Both alignments were added regardless. During preliminary comparisons, it was observed that 17 out of 712 amplicons were detected in the shotgun metagenomic library but not in the corresponding amplicon database. Most of these were detected at very low abundances, however seq86424 was an exception that comprised 24% and 20% of the 16S tags collected at 13 & 15 meters. These unmatched counts were dropped from the composition vector as they are false positives generated during the alignment process.

Amplicon alignments collected in the reverse and forward paired libraries were combined as the average of their relative abundances. This approach was also used for combining replicate samples collected in the amplicon dataset. The Pearson correlation between combined composition vectors was usually > 0.95 with the lowest few showing >0.74. Libraries from 11 matching depths were used in the comparative analysis.

The distances between abundances in amplicon libraries and those in shotgun libraries were calculated using three different normalization methods (L1, L2, or center-log ratios) in conjunction with seven different measures of beta-diversity, including Euclidean, Manhattan, and Bray-Curtis. For vectors that underwent the center-log ratio transform, different negative powers of ten were also tested as the pseudo-count. As the two sources of data are fundamentally different, the transformations were allowed to differ between data sets. Altogether 700 unique trials were conducted.

Ideally, the distance between the counts of a unique sequence observed in a set of amplicon libraries versus a set of shotgun metagenomic libraries derived from the same source material should be 0. By contrast, abundance vectors from each data set were selected at random without replacement and the distance between them measured in order to have a realistic null model. These two sets of distances were then compared by calculating the negative difference in their means, the negative difference in their medians, and the negative log of a p-value generated using t-test for paired samples. The results across these different performance measures were mostly consistent, but in order to obtain an objective winner, the three measures of difference metrics were ranked in ascending order and the three columns of ranks were summed. The resulting meta-rank allowed clear between trial conditions.

The distance measure that allowed for the greatest separation between the set of paired abundances vectors and randomly selected pairings was the cosine distance, followed by the correlation distance. The center log ratio using smaller negative powers of ten performed the best on the amplicon libraries and the L1 and the L2 norm worked equally well on the shotgun metagenomic counts.

**Metagenomic Binning Procedure**

The requisite numerical information recommended by the Genomic Standards Consortium for metagenome-assembled genome (1) is shown in Table X. The following approaches apply to all bins derived. Taxonomic classification was performed using the taxator-tk algorithm (). Reassembly of bins and initial co-assembly was performed using SPAdes 3.11(). The initial assembly was completed in metagenomic mode and reassembly was done for each bin in `careful` mode using the first pass contigs as `untrusted contigs` .

Contigs were binned according to their coverage & tetramer frequencies. A set of consensus bins were derived from the bins produced by the maxbin2, metabat2, and concoct algorithms. Completeness and contamination assessment were performed using the lineage workflow in CheckM. The bioinformatics pipeline from QC, to assembly, to binning, to refinement, to reassembly, and taxonomic classification was done within the metaWRAP software. Prokka () was used to facilitate gene calling and preliminary annotations. Within prokka, Prodigal, barrnap, and Aragorn v1.2 were used to call ORFs, rRNA & tRNA respectively (,,). All protein sequences were generated using Translation Table 11.

Metabolic Model Processes

, but additional protein annotations were conducted using `metabolic-hmms` collection provided by the Banfield lab (), the ` dbCAN-fam-HMMs.v6` collection provided by BioEnergy Science Center of the DOE (), and the KEGG BlastKOALA and GhostKOALA annotation web service ().

1. Robert M Bowers, Nikos C Kyrpides, Ramunas Stepanauskas, Miranda Harmon-Smith, Devin Doud, T B K Reddy, Frederik Schulz, Jessica Jarett, Adam R Rivers, Emiley A Eloe-Fadrosh, Susannah G Tringe, Natalia N Ivanova, Alex Copeland, Alicia Clum, Eric D Becraft, Rex R Malmstrom, Bruce Birren, Mircea Podar, Peer Bork, George M Weinstock, George M Garrity, Jeremy A Dodsworth, Shibu Yooseph, Granger Sutton, Frank O Glöckner, Jack A Gilbert, William C Nelson, Steven J Hallam, Sean P Jungbluth, Thijs J G Ettema, Scott Tighe, Konstantinos T Konstantinidis, Wen-Tso Liu, Brett J Baker, Thomas Rattei, Jonathan A Eisen, Brian Hedlund, Katherine D McMahon, Noah Fierer, Rob Knight, Rob Finn, Guy Cochrane, Ilene Karsch-Mizrachi, Gene W Tyson, Christian Rinke, The Genome Standards Consortium, Alla Lapidus, Folker Meyer, Pelin Yilmaz, Donovan H Parks, A Murat Eren, Lynn Schriml, Jillian F Banfield, Philip Hugenholtz & Tanja Woyke. (2017). Minimum information about a single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea. Nature Biotechnology 35(725–731) doi:10.1038/nbt.3893
2. Kanehisa, M., Sato, Y., and Morishima, K. (2016) BlastKOALA and GhostKOALA: KEGG tools for functional characterization of genome and metagenome sequences. J. Mol. Biol. 428, 726-731 (http://www.kegg.jp/ghostkoala/)